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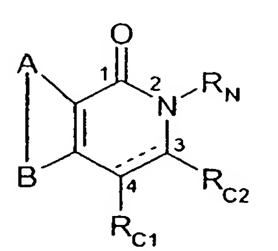
(54) Title: ISOQUINOLINONE DERIVATIVES AS PARP INHIBITORS



(I)

(57) Abstract: The use of a compound of the formula (I) and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein A and B together represent an optionally substituted, fused aromatic ring, the dotted line between the 3 and 4 positions indicates the optional presence of a double bond, at least one of R_{C1} and R_{C2} is independently represented by $-L-R_L$, and if one of R_{C1} and R_{C2} is not represented by $-L-R_L$, then that group is H, where L is of formula: $-(CH_2)_{n1}-Q_{n2}-(CH_2)_{n3}-$ wherein n_1, n_2 and n_3 are each selected from 0, 1, 2 and 3, the sum of n_1, n_2 and n_3 is 1, 2 or 3 and each Q (if n2 is greater than 1) is selected from O, S, NR_3, C(=O), or $-CR_1R_2-$, where R_1 and R_2 are independently selected from hydrogen, halogen or optionally substituted C_{1-7} alkyl, or may together with the carbon atom to which they are attached form a C_{3-7} cyclic alkyl group, which may be saturated (a C_{3-7} cycloalkyl group) or unsaturated (a C_{3-7} cycloalkenyl group), or one of R_1 and R_2 may be attached to an atom in R_L to form an unsaturated C_{3-7} cycloalkenyl group which comprises the carbon atoms to which R_1 and R_2 are attached in Q, $-(CH_2)_{n3}-$ (if present) and part of R_L, and where R_3 is selected from H or C_{1-7} alkyl, and R_L is selected from optionally substituted C_{3-20} heterocyclyl, C_{5-20} aryl and carbonyl, and R_N is selected from hydrogen, optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, hydroxy, ether, nitro, amino, thioether, sulfoxide and sulfone.

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ISOQUINOLINONE DERIVATIVES AS PARP INHIBITORS

The present invention relates to isoquinolinone derivatives, and their use as pharmaceuticals. In particular, the present invention relates to the use of these compounds to inhibit the activity of the enzyme poly (ADP-ribose)polymerase, also known as poly(ADP-ribose)synthase and poly ADP-ribosyltransferase, and commonly referred to as PARP.

The mammalian enzyme PARP (a 113-kDa multidomain protein) has been implicated in the signalling of DNA damage through its ability to recognize and rapidly bind to DNA single or double strand breaks (D'Amours et al, 1999, Biochem. J. 342: 249-268).

Several observations have led to the conclusion that PARP participates in a variety of DNA-related functions including gene amplification, cell division, differentiation, apoptosis, DNA base excision repair and also effects on telomere length and chromosome stability (d'Adda di Fagagna et al, 1999, Nature Gen., 23(1): 76-80).

Studies on the mechanism by which PARP modulates DNA repair and other processes has identified its importance in the formation of poly (ADP-ribose) chains within the cellular nucleus (Althaus, F.R. and Richter, C., 1987, ADP-Ribosylation of Proteins: Enzymology and Biological Significance, Springer-Verlag, Berlin). The DNA-bound, activated PARP utilizes NAD to synthesize poly (ADP-ribose) on a variety of nuclear target proteins, including topoisomerase, histones and PARP itself (Rhun et al, 1998, Biochem. Biophys. Res. Commun., 245: 1-10)

Poly (ADP-ribosyl)ation has also been associated with malignant transformation. For example, PARP activity is higher in the isolated nuclei of SV40-transformed fibroblasts, while both leukemic cells and colon cancer cells show higher enzyme activity than the equivalent normal leukocytes and colon mucosa (Miwa et al, 1977, Arch. Biochem. Biophys. 181: 313-321; Burzio et al, 1975, Proc. Soc. Exp. Bioi. Med. 149: 933-938; and Hirai et al, 1983, Cancer Res. 43: 3441-3446).

A number of low-molecular-weight inhibitors of PARP have been used to elucidate the functional role of poly (ADP-ribosyl)ation in DNA repair. In cells treated with alkylating agents, the inhibition of PARP leads to a marked increase in DNA-strand breakage and cell killing (Durkacz et al, 1980, Nature 283: 593-596; Berger, N.A., 1985, Radiation Research, 101: 4-14).

Subsequently, such inhibitors have been shown to enhance the effects of radiation response by suppressing the repair of potentially lethal damage (Ben-Hur et al, 1984, British Journal of Cancer, 49 (Suppl. VI): 34-42; Schlicker et al, 1999, Int. J. Radiat. Bioi., 75: 91-100). PARP inhibitors have been reported to be effective in radio sensitising hypoxic tumour cells (US 5,032,617; US 5,215,738 and US 5,041,653).

Furthermore, PARP knockout (PARP -/-) animals exhibit genomic instability in response to alkylating agents and γ-irradiation (Wang et al, 1995, Genes Dev., 9: 509-520; Menissier de Murcia et al, 1997, Proc. Natl. Acad. Sci. USA, 94: 7303-7307).

A role for PARP has also been demonstrated in certain vascular diseases, septic shock, ischaemic injury and neurotoxicity (Cantoni et al, 1989, Biochim. Biophys. Acta, 1014: 1-7; Szabo, et al, 1997, J. Clin.Invest., 100: 723-735). Oxygen radical DNA damage that leads to strand breaks in DNA, which are subsequently recognised by PARP, is a major contributing factor to such disease states as shown by PARP inhibitor studies (Cosi et al, 1994, J. Neurosci. Res., 39: 38-46; Said et al, 1996, Proc. Natl. Acad. Sci. U.S.A., 93: 4688-4692). More recently, PARP has been demonstrated to play a role in the pathogenesis of haemorrhagic shock (Liaudet et al, 2000, Proc. Natl. Acad. Sci. U.S.A., 97(3): 10203-10208).

It has also been demonstrated that efficient retroviral infection of mammalian cells is blocked by the inhibition of PARP activity. Such inhibition of recombinant retroviral vector infections was shown to occur in various different cell types (Gaken et al, 1996, J. Virology, 70(6): 3992-4000). Inhibitors of PARP have thus been developed for the use in anti-viral therapies and in cancer treatment (WO91/18591).

Moreover, PARP inhibition has been speculated to delay the onset of aging characteristics in human fibroblasts (Rattan and Clark, 1994, Biochem. Biophys. Res. Comm., 201 (2): 665-672). This may be related to the role that PARP plays in controlling telomere function (d'Adda di Fagagna et al, 1999, Nature Gen., 23(1): 76-80).

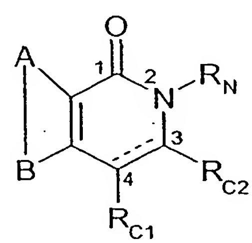
EP 0 355 750 discloses classes of 5-substituted isoquinolinones and dihydroisoquinolinones as PARP inhibitors. Exemplified substituents on the nitrogen

containing ring, at the 3 and/or 4 position, include methyl, phenyl, bromo or amino.

WO 99/11624 discloses a number of PARP inhibitors, amongst which are some isoquinolinone derivatives.

The present inventors have now discovered that further derivatives of isoquinolinone and dihydroisoquinolinone and related compounds act as PARP inhibitors.

Accordingly, the first aspect of the present invention provides for the use of compounds of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein:
A and B together represent an optionally substituted, fused aromatic ring;
the dotted line between the 3 and 4 positions indicates the optional presence of a double bond;
at least one of R_{C1} and R_{C2} is independently represented by -L-R_L, and if one of R_{C1} and R_{C2} is not represented by -L-R_L, then that group is H, where L is of formula:

$-(CH_2)_{n1}-Q_{n2}-(CH_2)_{n3}-$

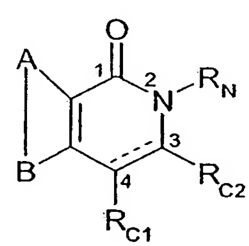
wherein n_1, n_2 and n_3 are each selected from 0, 1, 2 and 3, the sum of n_1, n_2 and n_3 is 1, 2 or 3 and each Q (if n2 is greater than 1) is selected from O, S, NR_3, C(=O), or $-CR_1R_2-$, where R_1 and R_2 are independently selected from hydrogen, halogen or optionally substituted C_{1-7} alkyl, or may together with the carbon atom to which they are attached form a C_{3-7}

cyclic alkyl group, which may be saturated (a C_{3-7} cycloalkyl group) or unsaturated (a C_{3-7} cycloalkenyl group), or one of R_1 and R_2 may be attached to an atom in R_L to form an unsaturated C_{3-7} cycloalkenyl group which comprises the carbon atoms to which R_1 and R_2 are attached in Q, $-(CH_2)_{n3}-$ (if present) and part of R_L, and where R_3 is selected from H or C_{1-7} alkyl; and

R_L is selected from optionally substituted C_{3-20} heterocyclyl, C_{5-20} aryl and carbonyl; and

R_N is selected from hydrogen, optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, hydroxy, ether, nitro, amino, thioether, sulfoxide and sulfone.

A second aspect of the invention provides compounds of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, wherein:

A and B together represent an optionally substituted, fused aromatic ring;

the dotted line between the 3 and 4 positions indicates the optional presence of a double bond;

one of R_{c1} and R_{c2} is $-CH_2-R_L$, and the other of R_{c1} and R_{c2} is H;

R_L is optionally substituted phenyl; and

R_N is hydrogen.

A third aspect of the present invention provides pharmaceutical compositions comprising a compound of the second aspect and a pharmaceutically acceptable carrier or diluent.

A fourth aspect of the present invention provides the use of a compound of the second aspect in a method of treatment of the human or animal body.

A further aspect of the invention provides for the use of compounds as defined in the first aspect of the invention in the preparation of a medicament for the treatment of: vascular disease; septic shock; ischaemic injury; neurotoxicity; haemorrhagic shock; viral infection; or diseases ameliorated by the inhibition of PARP.

A further aspect of the invention provides for the use of compounds as defined in the first aspect of the invention in the preparation of a medicament for use as an adjunct in cancer therapy or for potentiating tumour cells for treatment with ionising radiation or chemotherapeutic agents.

Definitions

The term "aromatic ring" is used herein in the conventional sense to refer to a cyclic aromatic structure, that is, a cyclic structure having delocalised π-electron orbitals.

The aromatic ring fused to the main core, i.e. that formed by -A-B-, may bear further fused aromatic rings (resulting in, e.g. naphthyl or anthracenyl groups). The aromatic ring(s) may comprise solely carbon atoms, or may comprise carbon atoms and one or more heteroatoms, including but not

limited to, nitrogen, oxygen, and sulfur atoms. The aromatic ring(s) preferably have five or six ring atoms.

The aromatic ring(s) may optionally be substituted. If a substituent itself comprises an aryl group, this aryl group is not considered to be a part of the aryl group to which it is attached. For example, the group biphenyl is considered herein to be a phenyl group (an aryl group comprising a single aromatic ring) substituted with a phenyl group. Similarly, the group benzylphenyl is considered to be a phenyl group (an aryl group comprising a single aromatic ring) substituted with a benzyl group.

In one group of preferred embodiments, the aromatic group comprises a single aromatic ring, which has five or six ring atoms, which ring atoms are selected from carbon, nitrogen, oxygen, and sulfur, and which ring is optionally substituted. Examples of these groups include benzene, pyrazine, pyrrole, thiazole, isoxazole, and oxazole. 2-Pyrone can also be considered to be an aromatic ring, but is less preferred.

If the aromatic ring has six atoms, then preferably at least four, or even five or all, of the ring atoms are carbon. The other ring atoms are selected from nitrogen, oxygen and sulphur, with nitrogen and oxygen being preferred. Suitable groups include a ring with: no hetero atoms (benzene); one nitrogen ring atom (pyridine); two nitrogen ring atoms (pyrazine, pyrimidine and pyridazine); one oxygen ring atom (pyrone); and one oxygen and one nitrogen ring atom (oxazine).

If the aromatic ring has five ring atoms, then preferably at least three of the ring atoms are carbon. The remaining

ring atoms are selected from nitrogen, oxygen and sulphur. Suitable rings include a ring with: one nitrogen ring atom (pyrrole); two nitrogen ring atoms (imidazole, pyrazole); one oxygen ring atom (furan); one sulphur ring atom (thiophene); one nitrogen and one sulphur ring atom (isothiazole or thiazole); and one nitrogen and one oxygen ring atom (isoxazole or oxazole).

The aromatic ring may bear one or more substituent groups at any available ring position. These substituents are selected from halo, nitro, hydroxy, ether, thiol, thioether, amino, C_{1-7} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl. The aromatic ring may also bear one or more substituent groups which together form a ring. In particular these may be of formula $-(CH_2)_m-$ or $-O-(CH_2)_p-O-$, where m is 2, 3, 4 or 5 and p is 1, 2 or 3.

C_{1-7} alkyl: The term "C_{1-7} alkyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a C_{1-7}hydrocarbon compound having from 1 to 7 carbon atoms, which may be aliphatic or alicyclic, or a combination thereof, and which may be saturated, partially unsaturated, or fully unsaturated.

Examples of (unsubstituted) saturated linear C_{1-7} alkyl groups include, but are not limited to, methyl, ethyl, *n*-propyl, *n*-butyl, and *n*-pentyl (amyl).

Examples of (unsubstituted) saturated branched C_{1-7} alkyl groups include, but are not limited to, *iso*-propyl, *iso*-butyl, *sec*-butyl, *tert*-butyl, and *neo*-pentyl.

Examples of saturated alicyclic (carbocyclic) C_{1-7} alkyl groups (also referred to as "C_{3-7} cycloalkyl" groups)

include, but are not limited to, unsubstituted groups such as cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl, as well as substituted groups (e.g. groups which comprise such groups), such as methylcyclopropyl, dimethylcyclopropyl, methylcyclobutyl, dimethylcyclobutyl, methylcyclopentyl, dimethylcyclopentyl, methylcyclohexyl, cyclopropylmethyl and cyclohexylmethyl.

Examples of (unsubstituted) unsaturated C_{1-7} alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C_{2-7} alkenyl" groups) include, but are not limited to, ethenyl (vinyl, $-CH=CH_2$), 2-propenyl (allyl, $-CH_2-CH=CH_2$), isopropenyl ($-C(CH_3)=CH_2$), butenyl, pentenyl, and hexenyl.

Examples of (unsubstituted) unsaturated C_{1-7} alkyl groups which have one or more carbon-carbon triple bonds (also referred to as "C_{2-7} alkynyl" groups) include, but are not limited to, ethynyl (ethinyl) and 2-propynyl (propargyl).

Examples of unsaturated alicyclic (carbocyclic) C_{1-7} alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C_{3-7} cycloalkenyl" groups) include, but are not limited to, unsubstituted groups such as cyclopropenyl, cyclobutenyl, cyclopentenyl, and cyclohexenyl, as well as substituted groups (e.g. groups which comprise such groups) such as cyclopropenylmethyl and cyclohexenylmethyl.

C_{3-20} heterocyclyl: The term "C_{3-20} heterocyclyl," as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a non-aromatic C_{3-20} heterocyclic compound, said compound having one ring, or two or more rings (e.g. spiro, fused, bridged), and having from

3 to 20 ring atoms, of which from 1 to 10 are ring heteroatoms, and wherein at least one of said ring(s) is a heterocyclic ring. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms. "C_{3-20}" denotes ring atoms, whether carbon atoms or heteroatoms.

Examples of C_{3-20} heterocyclyl groups having one nitrogen ring atom include, but are not limited to, those derived from aziridine, azetidine, azetine, pyrrolidine, pyrroline, piperidine, dihydropyridine, tetrahydropyridine, and dihydropyrrole (azoline).

Examples of C_{3-20} heterocyclyl groups having one oxygen ring atom include, but are not limited to, those derived from oxirane, oxetane, oxolane (tetrahydrofuran), oxole (dihydrofuran), oxane (tetrahydropyran), dihydropyran, and pyran. Examples of substituted C_{3-20} heterocyclyl groups include sugars, in cyclic form, for example, furanoses and pyranoses, including, for example, ribose, lyxose, xylose, galactose, sucrose, fructose, and arabinose.

Examples of C_{3-20} heterocyclyl groups having one sulfur ring atom include, but are not limited to, those derived from thiolane (tetrahydrothiophene, thiane) and tetrahydrothiopyran.

Examples of C_{3-20} heterocyclyl groups having two oxygen ring atoms include, but are not limited to, those derived from dioxane, for example 1,3-dioxane and 1,4-dioxane.

Examples of C_{3-20} heterocyclyl groups having two nitrogen ring atoms include, but are not limited to, those derived from diazolidine (pyrazolidine), pyrazoline, imidazolidine, imidazoline, and piperazine.

Examples of C_{3-20} heterocyclyl groups having one nitrogen ring atom and one oxygen ring atom include, but are not limited to, those derived from tetrahydrooxazole, dihydrooxazole, tetrahydroisoxazole, dihydroiosoxazole, morpholine, tetrahydrooxazine, dihydrooxazine, and oxazine.

Examples of C_{3-20} heterocyclyl groups having one oxygen ring atom and one sulfur ring atom include, but are not limited to, those derived from oxathiolane and oxathiane.

Examples of C_{3-20} heterocyclyl groups having one nitrogen ring atom and one sulfur ring atom include, but are not limited to, those derived from thiazoline, thiazolidine, and thiomorpholine.

Other examples of C_{3-20} heterocyclyl groups include, but are not limited to, oxadiazine.

If the C_{3-20} heterocyclyl is substituted, the substituents are on carbon, or nitrogen (if present), atoms.

C_{5-20} aryl: The term "C_{5-20} aryl," as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of a C_{5-20} aromatic compound, said compound having one ring, or two or more rings (e.g. fused), and having from 5 to 20 ring atoms, and wherein at least one of said ring(s) is an aromatic ring. Preferably, each ring has from 5 to 7 ring atoms.

The ring atoms may be all carbon atoms, as in "carboaryl groups," in which case the group may conveniently be referred to as a "C_{5-20} carboaryl" group.

Examples of C_{5-20} aryl groups which do not have ring heteroatoms (i.e., C_{5-20} carboaryl groups) include, but are not limited to, those derived from benzene (i.e., phenyl) (C_6), naphthalene (C_{10}), anthracene (C_{14}), phenanthrene (C_{14}), and pyrene (C_{16}).

Alternatively, the ring atoms may include one or more heteroatoms, including but not limited to oxygen, nitrogen, and sulfur, as in "heteroaryl groups." In this case, the group may conveniently be referred to as a "C_{5-20} heteroaryl" group, wherein "C_{5-20}" denotes ring atoms, whether carbon atoms or heteroatoms. Preferably, each ring has from 5 to 7 ring atoms, of which from 0 to 4 are ring heteroatoms.

Examples of C_{5-20} heteroaryl groups include, but are not limited to, C_5 heteroaryl groups derived from furan (oxole), thiophene (thiole), pyrrole (azole), imidazole (1,3-diazole), pyrazole (1,2-diazole), triazole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, oxatriazole, and tetrazole; and C_6 heteroaryl groups derived from isoxazine, pyridine (azine), pyridazine (1,2-diazine), pyrimidine (1,3-diazine; e.g. cytosine, thymine, uracil), pyrazine (1,4-diazine), and triazine.

The heteroaryl group may be bonded via a carbon or hetero ring atom.

Examples of C_{5-20} heteroaryl groups which comprise fused rings, include, but are not limited to, C_9 heteroaryl groups derived from benzofuran, isobenzofuran, benzothiophene, indole, isoindole; C_{10} heteroaryl groups derived from quinoline, isoquinoline, benzodiazine, pyridopyridine; C_{14} heteroaryl groups derived from acridine and xanthene.

The above C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7}alkoxy group), a C_{3-20} heterocyclyl group (also referred to as a C_{3-20} heterocyclyloxy group), or a C_{5-20} aryl group (also referred to as a C_{5-20} aryloxy group), preferably a C_{1-7} alkyl group.

Nitro: $-NO_2$.

Cyano (nitrile, carbonitrile): -CN.

Carbonyl: a group of structure -C(=O)-, which includes acyl, carboxy, ester and amido.

Acyl (keto): -C(=O)R, wherein R is an acyl substituent, for example, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylacyl or C_{1-7} alkanoyl), a C_{3-20} heterocyclyl group (also referred to as C_{3-20} heterocyclylacyl), or a C_{5-20} aryl group (also referred to as C_{5-20} arylacyl), preferably a C_{1-7} alkyl group. Examples of acyl groups include, but are not limited to, $-C(=O)CH_3$ (acetyl), $-C(=O)CH_2CH_3$ (propionyl), $-C(=O)C(CH_3)_3$ (pivaloyl), and -C(=O)Ph (benzoyl, phenone).

Carboxy (carboxylic acid): -COOH.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): $-C(=O)OR$, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, $-C(=O)OCH_3$, $-C(=O)OCH_2CH_3$, $-C(=O)OC(CH_3)_3$, and $-C(=O)OPh$.

Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which R^1 and R^2, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

Amino: $-NR^1R^2$, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, or, in the case of a "cyclic" amino group, R^1 and R^2, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHCH(CH_3)_2$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, and $-NHPh$. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, perhydrodiazepino, morpholino, and thiomorpholino. The cyclic amino groups may be substituted on their ring by any of the substituents defined here, for example carboxy, carboxylate and amido. A particular form of amino group is where one of R^1 and R^2 is a sulfone

($-S(=O)_2R$), where R is a sulfone substituent, and this group can be termed a sulfonamido group. Examples of sulfonamido groups include, but are not limited to, $-NHS(=O)_2CH_3$, $-NHS(=O)_2Ph$ and $-NHS(=O)_2C_6H_4F$.

Acylamido (acylamino): $-NR^1C(=O)R^2$, wherein R^1 is an amide substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7}alkyl group, most preferably H, and R^2 is an acyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of acylamido groups include, but are not limited to, $-NHC(=O)CH_3$, $-NHC(=O)CH_2CH_3$, and $-NHC(=O)Ph$. One particular form of acylamido group is where R^2 is an amino group ($-NR^3R^4$), where R^3 and R^4 are independently amino substituents, and this group can be termed an ureido group. Examples of ureido groups include, but are not limited to $-NHC(=O)NHCH_3$, $-NHC(=O)NHCH_2CH_3$, and $-NHC(=O)NHPh$.

Acyloxy (reverse ester): $-OC(=O)R$, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Example of acyloxy groups include, but are not limited to, $-OC(=O)CH_3$ (acetoxy), $-OC(=O)CH_2CH_3$, $-OC(=O)C(CH_3)_3$, $-OC(=O)Ph$, $-OC(=O)CH_4F$, and $-OC(=O)CH_2Ph$.

Thiol : $-SH$.

Thioether (sulfide): $-SR$, wherein R is a thioether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkylthio group), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of C_{1-7} alkylthio groups include, but are not limited to, $-SCH_3$ and $-SCH_2CH_3$.

Sulfoxide (sulfinyl): -S(=O)R, wherein R is a sulfoxide substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfoxide groups include, but are not limited to, $-S(=O)CH_3$ and $-S(=O)CH_2CH_3$.

Sulfone (sulfonyl): $-S(=O)_2R$, wherein R is a sulfone substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfone groups include, but are not limited to, $-S(=O)_2CH_3$ (methanesulfonyl, mesyl), $-S(=O)_2CF_3$, $-S(=O)_2CH_2CH_3$, and 4-methylphenylsulfonyl (tosyl).

As mentioned above, the groups that form the above listed substituent groups, e.g. C_{1-7} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl, may themselves be substituted. Thus, the above definitions cover substituent groups which are substituted.

Substituents Form a Ring

It is possible that a substituent on a ring which forms part of R_{C1} and a substituent on the fused aromatic ring (represented by -A-B-), may together form an intra ring link, thus forming a further cyclic structure in the compound.

The substituent on the aromatic ring that forms the intra ring link is preferably on the atom adjacent the central moiety (i.e. at the α-position).

The substituent on R_{C1} that forms the intra ring link is preferably on the atom which is one atom away from the atom which is bound to the central moiety.

The link between the two rings may be a single bond, or may be of the formula:

$-(CH_2)_{n1'}-Q'_{n2'}-(CH_2)_{n3'}-$

wherein n1', n2' and n3' are each selected from 0, 1, 2 and 3 and the sum of n1', n2' and n3' is less than or equal to 3. Each Q' (if n2' is greater than 1) is selected from O, S, NR'_3, C(=O), or $-CR'_1R'_2-$, where R'_1 and R'_2 are independently selected from hydrogen, halogen or optionally substituted C_{1-7} alkyl, or may together with the carbon atom to which they are attached form a C_{3-7} cyclic alkyl group, which may be saturated (a C_{3-7} cycloalkyl group) or unsaturated (a C_{3-7} cycloalkenyl group), and where R'_3 is selected from H or C_{1-7} alkyl.

Further Preferences

It is preferred that there is a double bond present between the third and fourth positions of the compound.

It is also preferred that only one of R_{C1} and R_{C2} is represented by $-L-R_L$, and the other of R_{C1} and R_{C2} is H. The preferences for L and R_L expressed below may be different for R_{C1} and R_{C2}.

The fused aromatic ring(s) represented by -A-B- preferably consist of solely carbon ring atoms, and thus may be benzene, naphthalene, and is more preferably benzene. As described above, these rings may be substituted, but in some embodiments are preferably unsubstituted.

R_N is preferably selected from hydrogen, and C_{1-7} alkyl, which may be substituted or unsubstituted. In one embodiment, R_N is preferably C_{1-3} alkyl, which may be substituted, for example by a C_{5-20} heterocyclic group.

Suitable such groups include cyclic amino groups such as piperidino or morpholino. In another embodiment, R_N is preferably H.

In L, it is preferred that each Q (if n2 is greater than 1) is selected from O, S, NH or C(=O).

L is preferably of formula:
$-(CH_2)_{n1}-Q_{n2}-$, where n1 is selected from 0, 1, 2 and 3 and n2 is selected from 0 and 1 (where the sum of n1 and n2 is 1, 2 or 3), and more preferably n1 is 1 or 2. The more preferred options for L are $-CH_2-$ or $-C_2H_4-$, with $-C_2H_4-$ being the most preferred for R_{C2} and $-CH_2-$ being the most preferred for R_{C1}.

If Q in L is $-CR_1R_2-$, then n2 is preferably 1. In one embodiment, R_1 is optionally substituted C_{1-7} alkyl and R_2 is hydrogen. R_1 is more preferably optionally substituted C_{1-4} alkyl, and most preferably unsubstituted C_{1-4} alkyl. In another embodiment, R_1 and R_2, together with the carbon atom to which they are attached, form a saturated C_{3-7} cyclic alkyl group, more preferably a C_{5-7} cyclic alkyl group. In a further embodiment, R_1 is attached to an atom in R_L to form an unsaturated C_{3-7} cycloalkenyl group, more preferably a C_{5-7} cycloalkenyl group, which comprises the carbon atoms to which R_1 and R_2 are attached in Q, $-(CH_2)_{n3}-$ (if present) and part of R_L, and R_2 is hydrogen.

R_L is preferably C_{5-20} aryl, and more preferably a benzene ring, naphthalene, pyridine, 1,3-benzodioxole or furan.

When R_L is a benzene ring, it is preferably substituted. The one or more substituents may be selected from: C_{1-7} alkyl, more preferably methyl, CF_3; C_{5-20} aryl; C_{3-20} heterocyclyl; halo, more preferably fluoro; hydroxy; ether,

more preferably methoxy, phenoxy, benzyloxy, and cyclopentoxy; nitro; cyano; carbonyl groups, such as carboxy, ester and amido; amino (including sulfonamido), more preferably $-NH_2$, -NHPh, and cycloamino groups, such as morpholino; acylamido, including ureido groups, where the acyl or amino substituent is preferably phenyl, which itself is optionally fluorinated; acyloxy; thiol; thioether; sulfoxide; sulfone.

In one group of embodiments, fluoro is particularly preferred as a substituent, along with substituents containing a phenyl, or fluorinated phenyl, component.

Preferred substituents of the benzene ring, when R_L is phenyl, include:

(i) acylamido, wherein the amide substituent is selected from C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, more preferably C_{1-7} alkyl and C_{5-20} aryl, which groups are optionally further substituted. The optional substituents may be selected from any of those listed above, but those of particular interest include C_{1-7} alkyl and C_{5-20} aryl groups, halo, ether, thioether and sulfone groups;

(ii) ureido, where one amine substituent is preferably hydrogen, and the other is selected from C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, more preferably C_{1-7} alkyl and C_{5-20} aryl, which groups are optionally further substituted. The optional substituents may be selected from any one of those listed above, but those of particular interest include C_{1-7} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups, halo and ether groups;

(iii)sulfonamino, wherein the amine substituent is preferably hydrogen and the sulfone substituent is selected from C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, more preferably C_{1-7} alkyl and C_{5-20} aryl, which groups are

optionally further substituted. The optional substituents may be selected from any of those listed above, but those of particular interest include C_{5-20} aryl groups and acylamido groups;

(iv) acyloxy, wherein the acyloxy substituent is selected from C_{1-7} alkyl, C_{3-20} heterocyclyl, and C_{5-20} aryl, more preferably C_{1-7} alkyl and C_{5-20} aryl, which groups are optionally further substituted. The optional substituents may be selected from any of those listed above, but those of particular interest include C_{1-7} alkyl and C_{5-20} aryl groups, halo, ether, thioether, sulfone and nitro groups.

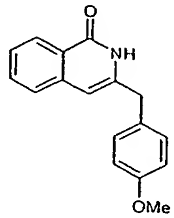
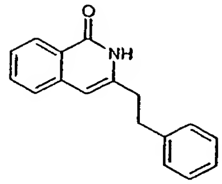
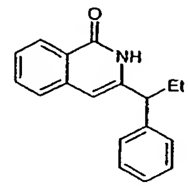
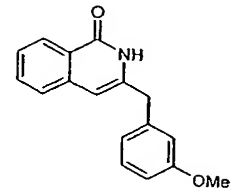
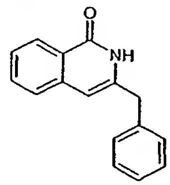
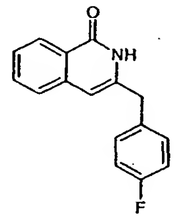
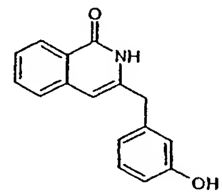
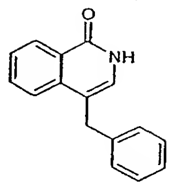
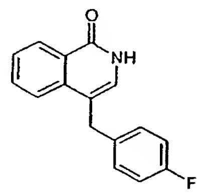
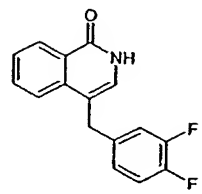
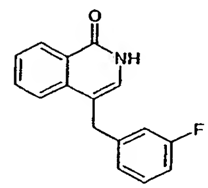
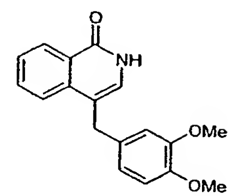
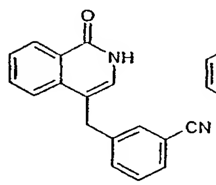
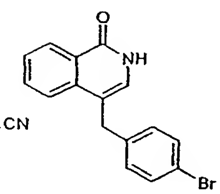
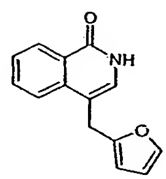
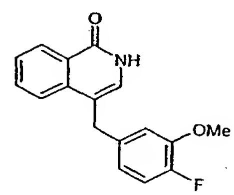
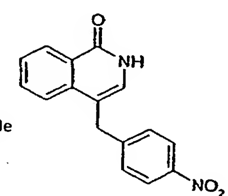
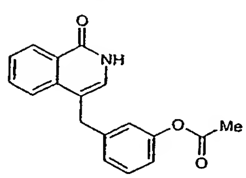
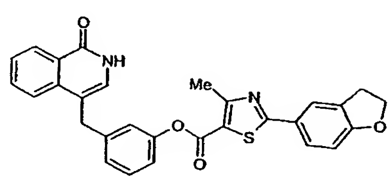
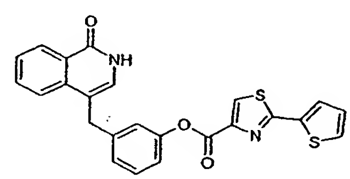
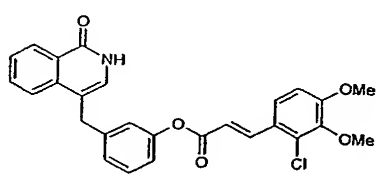
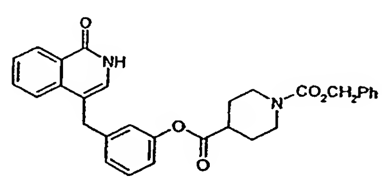
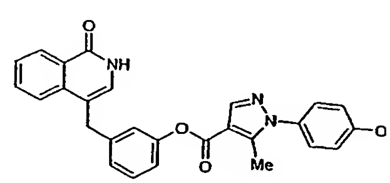
If A and B together represent a substituted fused aromatic ring, it is preferred that the substituent does not form an intra ring link with a substituent on a ring which forms part of R_c. Substituents in the five position are particularly preferred.

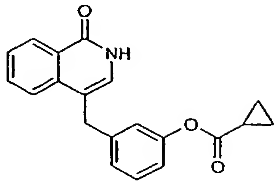
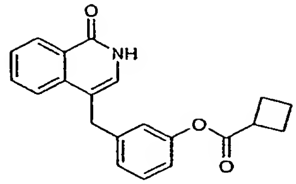
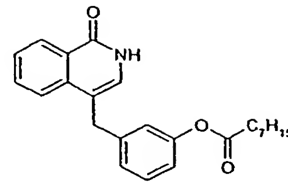
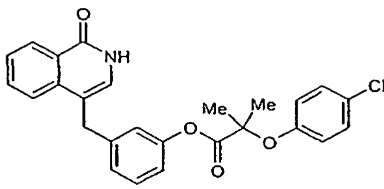
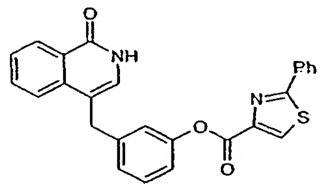
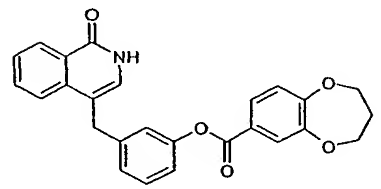
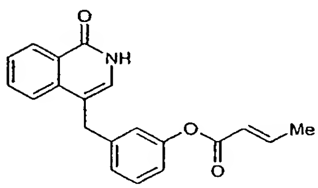
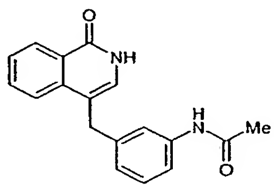
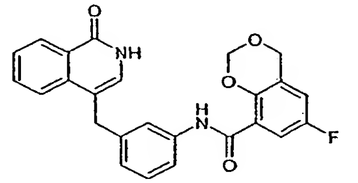
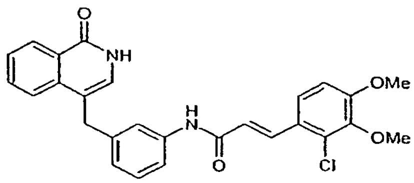
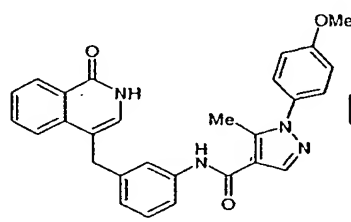
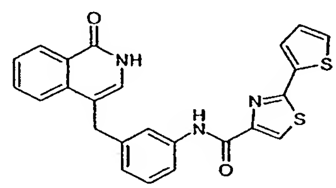
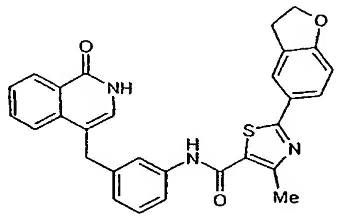
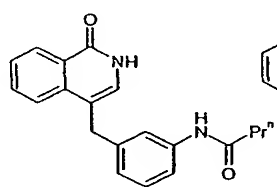
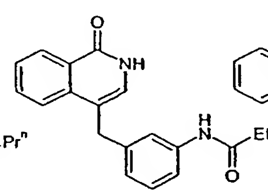
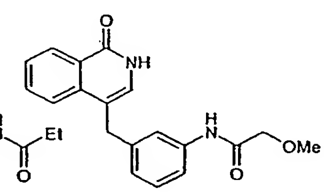
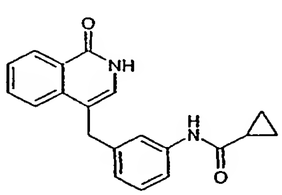
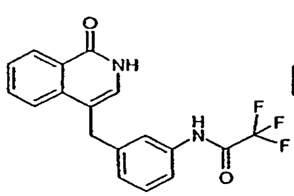
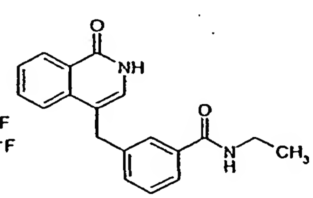
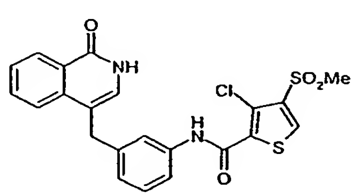
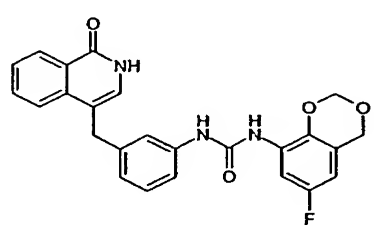
In particular, when R_L is $-CH_2$-phenyl, the phenyl group is preferably substituted.

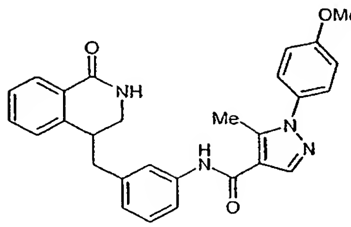
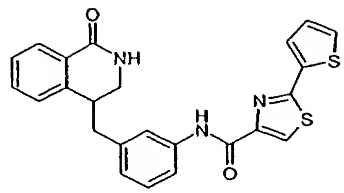
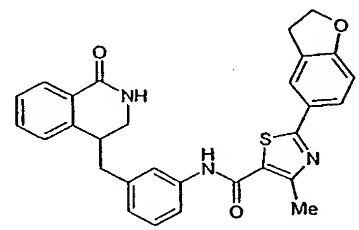
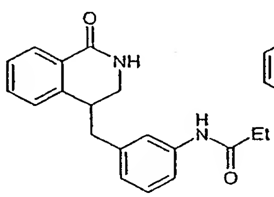
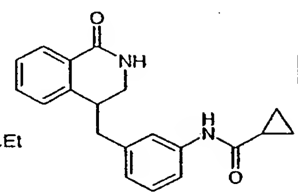
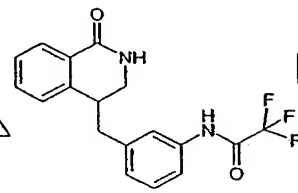
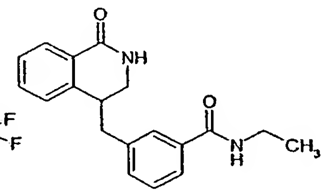
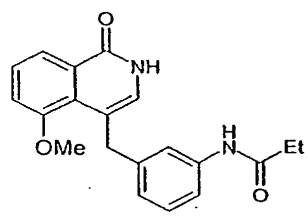
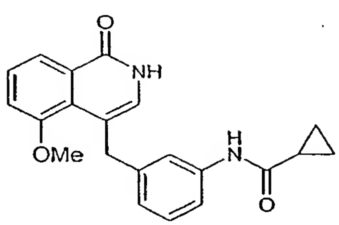
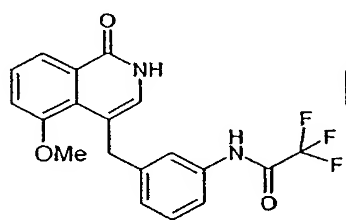
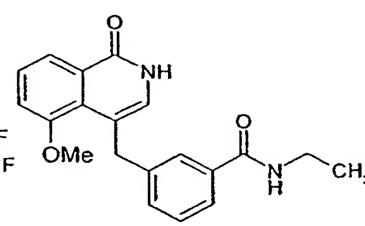
Where appropriate, the above preferences may be taken in combination with each other.

Preferred Compounds

The following compounds are preferred embodiments of the first aspect of the invention:

Includes Other Forms

Included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form ($-COO^-$), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form ($-N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic

form ($-O^-$), a salt or solvate thereof, as well as conventional protected forms of a hydroxyl group.

Isomers, Salts, Solvates, Protected Forms, and Prodrugs

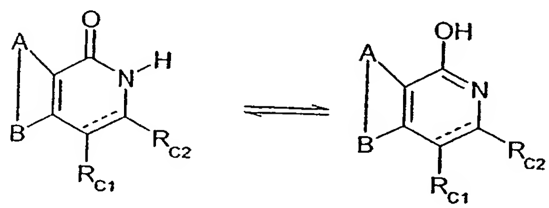
Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, *cis*- and *trans*-forms; *E*- and *Z*-forms; *c*-, *t*-, and *r*- forms; *endo*- and *exo*-forms; *R*-, *S*-, and *meso*-forms; *D*- and *L*-forms; *d*- and *l*-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

If the compound is in crystalline form, it may exist in a number of different polymorphic forms.

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers," as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, $-OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $-CH_2OH$. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g. C_{1-7}alkyl includes *n*-propyl and *iso*-propyl; butyl includes *n*-, *iso*-, *sec*-, and *tert*-butyl; methoxyphenyl includes *ortho*-, *meta*-, and *para*-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol, imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.

Particularly relevant to the present invention is the tautomeric pair that exists when R_N is H, illustrated below:



Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^1H, ^2H (D), and ^3H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g. fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge et al., 1977, "Pharmaceutically Acceptable Salts," J. Pharm. Sci., Vol. 66, pp. 1-19.

For example, if the compound is anionic, or has a functional group which may be anionic (e.g. -COOH may be $-COO^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na+ and K+, alkaline earth cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{+3}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g. NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

If the compound is cationic, or has a functional group which may be cationic (e.g. $-NH_2$ may be $-NH_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous. Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: acetic, propionic, succinic, gycolic, stearic, palmitic, lactic, malic, pamoic,

tartaric, citric, gluconic, ascorbic, maleic, hydroxymaleic, phenylacetic, glutamic, aspartic, benzoic, cinnamic, pyruvic, salicyclic, sulfanilic, 2-acetyoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethanesulfonic, ethane disulfonic, oxalic, isethionic, valeric, and gluconic. Examples of suitable polymeric anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form," as used herein, pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions, that is, are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, Protective Groups in Organic Synthesis (T. Green and P. Wuts, Wiley, 1991).

For example, a hydroxy group may be protected as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH_3, -OAc).

For example, an aldehyde or ketone group may be protected as an acetal or ketal, respectively, in which the carbonyl group (>C=O) is converted to a diether (>C$(OR)_2$), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

For example, an amine group may be protected, for example, as an amide or a urethane, for example, as: a methyl amide (-NHCO-CH_3); a benzyloxy amide (-NHCO-O$CH_2C_6H_5$, -NH-Cbz); as a t-butoxy amide (-NHCO-OC$(CH_3)_3$, -NH-Boc); a 2-biphenyl-2-propoxy amide (-NHCO-OC$(CH_3)_2C_6H_4C_6H_5$, -NH-Bpoc), as a 9-fluorenylmethoxy amide (-NH-Fmoc), as a 6-nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec); or, in suitable cases, as an *N*-oxide (>NO•).

For example, a carboxylic acid group may be protected as an ester for example, as: an C_{1-7} alkyl ester (e.g. a methyl ester; a t-butyl ester); a C_{1-7} haloalkyl ester (e.g. a C_{1-7} trihaloalkyl ester); a triC_{1-7}alkylsilyl-C_{1-7}alkyl ester; or a C_{5-20} aryl-C_{1-7} alkyl ester (e.g. a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether ($-S-CH_2NHC(=O)CH_3$).

It may be convenient or desirable to prepare, purify, and/or handle the active compound in the form of a prodrug. The term "prodrug", as used herein, pertains to a compound which, when metabolised (e.g. *in vivo*), yields the desired active compound. Typically, the prodrug is inactive, or less active than the active compound, but may provide advantageous handling, administration, or metabolic properties.

For example, some prodrugs are esters of the active compound (e.g. a physiologically acceptable metabolically labile ester). During metabolism, the ester group (-C(=O)OR) is cleaved to yield the active drug. Such esters may be formed by esterification, for example, of any of the carboxylic acid groups (-C(=O)OH) in the parent compound, with, where appropriate, prior protection of any other reactive groups present in the parent compound, followed by deprotection if required. Examples of such metabolically labile esters include those wherein R is C_{1-7}alkyl (e.g. -Me, -Et); C_{1-7}aminoalkyl (e.g. aminoethyl; 2-(N,N-diethylamino)ethyl; 2-(4-morpholino)ethyl); and acyloxy-C_{1-7}alkyl (e.g. acyloxymethyl; acyloxyethyl; e.g. pivaloyloxymethyl; acetoxymethyl; 1-acetoxyethyl; 1-(1-methoxy-1-methyl)ethyl-carbonxyloxyethyl; 1-(benzoyloxy)ethyl; isopropoxy-carbonyloxymethyl; 1-isopropoxy-carbonyloxyethyl; cyclohexyl-carbonyloxymethyl; 1-cyclohexyl-carbonyloxyethyl; cyclohexyloxy-carbonyloxymethyl; 1-cyclohexyloxy-carbonyloxyethyl; (4-tetrahydropyranyloxy) carbonyloxymethyl; 1-(4-tetrahydropyranyloxy) carbonyloxyethyl; (4-tetrahydropyranyl)carbonyloxymethyl;

and 1-(4-tetrahydropyranyl)carbonyloxyethyl). Further suitable prodrug forms include phosphonate and glycolate salts.

Also, some prodrugs are activated enzymatically to yield the active compound, or a compound which, upon further chemical reaction, yields the active compound. For example, the prodrug may be a sugar derivative or other glycoside conjugate, or may be an amino acid ester derivative.

Acronyms

For convenience, many chemical moieties are represented using well known abbreviations, including but not limited to, methyl (Me), ethyl (Et), *n*-propyl (nPr), *iso*-propyl (iPr), *n*-butyl (nBu), *tert*-butyl (tBu), *n*-hexyl (nHex), cyclohexyl (cHex), phenyl (Ph), biphenyl (biPh), benzyl (Bn), naphthyl (naph), methoxy (MeO), ethoxy (EtO), benzoyl (Bz), and acetyl (Ac).

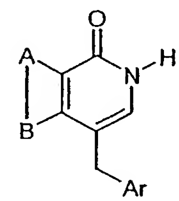
For convenience, many chemical compounds are represented using well known abbreviations, including but not limited to, methanol (MeOH), ethanol (EtOH), iso-propanol (i-PrOH), methyl ethyl ketone (MEK), ether or diethyl ether (Et_2O), acetic acid (AcOH), dichloromethane (methylene chloride, DCM), trifluoroacetic acid (TFA), dimethylformamide (DMF), tetrahydrofuran (THF), and dimethylsulfoxide (DMSO).

Synthesis

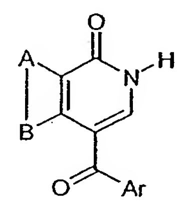
Compounds as described in the first aspect can be synthesised by a number of methods, examples of some of which are given below.

The following papers provide routes to compounds within the general class illustrated (where Ar = C_{5-20} aryl), and these papers are herein incorporated by reference.

I.W. Elliott and Y. Takekoshi, J. Heterocyclic Chem., 1976, ***13****, 597.*



K. Masayasu, I. Waki, Y. Deguchi, K. Amemiya and T. Maeda, Chem. Pharm. Bull., 1983, ***31****(4), 1277.*



The formed aromatic ring (represented by -A-B-) is usually derivatised before the main synthesis steps, and starting materials with the desired structure and substituent pattern are either commercially available or readily synthesised.

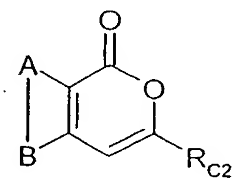
The main synthesis steps may lead to compounds where R_N is H. The possible substituents at this position can be added by the use of an appropriate electrophile with suitable reaction conditions.

Further derivatisation of the groups on R_{C1} and R_{C2} can be carried out using conventional methods.

<u>Synthesis of 3-substituted isoquinolinones</u>

Compounds of the present invention in which R_{C1} is H and R_{C2},

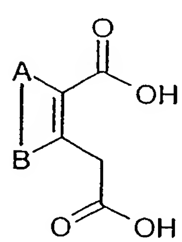
R_N, A and B are as defined in the first aspect and the bond joining positions 3 and 4 is a double bond, may be synthesised by reaction of a compound of Formula 1:



Formula 1

in which R_{C2}, A and B are as previously defined, with a compound of formula R_NNH_2, in which R_N is as previously defined, at a temperature in the range of 100-200°C, optionally in a sealed vessel so as to generate high pressure, optionally in the presence of a solvent, for example methanol.

Compounds of Formula 1 may be synthesised by reaction of a compound of Formula 2:



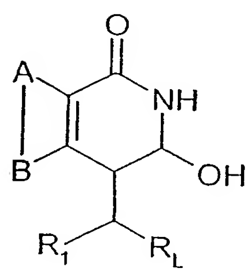
Formula 2

in which A and B are as defined above, with a compound of formula $R_{C2}COX$, in which R_{C2} is as previously defined and X is a leaving group, for example a halogen such as chlorine, at a temperature in the range of 100-250°C, optionally in the presence of a solvent, for example xylene.

Compounds of Formula 2 are commercially available or may be readily prepared by known methods.

Synthesis of 4-substituted isoquinolinones

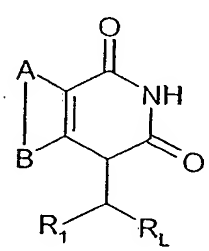
Compounds of the present invention in which R_{C1} is an arylalkyl group of formula R_LCHR_1- in which R_L and R_1 are as defined in the first aspect, R_{C2} and R_N are H and A and B are as defined in the first aspect and the bond joining positions 3 and 4 is a double bond, may be synthesised by reaction of a compound of Formula 3:



Formula 3

in which A, B, R_L and R_1 are as previously defined, with a dehydrating agent, for example toluene-4-sulphonic acid, at a temperature in the range of 20-150°C, optionally in the presence of a solvent, for example toluene.

Compounds of Formula 3 may be synthesised by reaction of a compound of Formula 4:

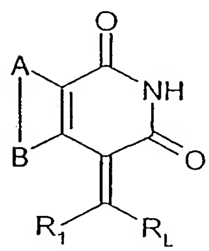


Formula 4

in which A, B, R_L and R_1 are as previously defined with a reducing agent, for example a source of hydride such as sodium borohydride, in a solvent, for example methanol, at a temperature in the range of -20°C to the boiling point of

the chosen solvent.

Compounds of Formula 4 may be synthesised by reduction of a compound of Formula 5:

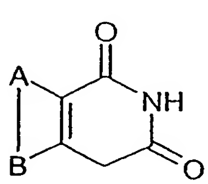


Formula 5

in which A, B, R_L and R_1 are as previously defined with a reducing agent, for example hydrogen, in the presence of an appropriate catalyst, for example palladium-on-carbon, in the presence of a solvent, for example methanol, at a temperature in the range of 20°C to the boiling point of the chosen solvent, optionally under increased pressure.

Compounds of Formula 3 may also be synthesised directly from Compounds of Formula 5 by reaction with a reducing agent, for example a source of hydride such as sodium borohydride, in a solvent, for example methanol, at a temperature in the range of -20°C to the boiling point of the chosen solvent.

Compounds of Formula 5 may be synthesised by reaction of a compound of Formula 6:



Formula 6

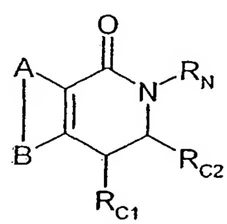
in which A and B are as previously defined, with a carbonyl compound of Formula R_LCOR_1 in which R_L and R_1 are as

previously defined, in the presence of a base, for example piperidine, optionally in the presence of a solvent, for example acetic acid, at a temperature in the range of 20°C to the boiling point of the chosen solvent.

Compounds of Formula 6 may be synthesised by reaction of a compound of Formula 2 with urea at a temperature in the range of 150-190°C.

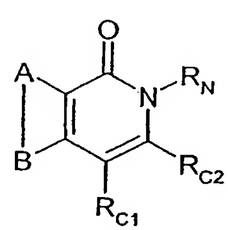
<u>Synthesis of 3- or 4-substituted 3,4-dihydroisoquinolones</u>

Compounds of the present invention in which the bond joining positions 3 and 4 is a single bond (i.e. Compounds of Formula 7):



Formula 7

in which R_{C1}, R_{C2}, R_N, A and B are as defined in the first aspect may be synthesised by reduction of Compounds of the present invention in which the bond joining positions 3 and 4 is a double bond (i.e. Compounds of Formula 8):



Formula 8

in which R_{C1}, R_{C2}, R_N, A and B are as defined in the first aspect with a reducing agent, for example hydrogen or ammonium

formate, in the presence of an appropriate catalyst, for example palladium-on-carbon or Raney Nickel, in the presence of a solvent, for example ethanol or acetic acid, at a temperature in the range of 20°C to the boiling point of the chosen solvent, optionally under increased pressure.

Use

The present invention provides active compounds, specifically, active in inhibiting the activity of PARP.

The term 'active', as used herein, pertains to compounds which are capable of inhibiting PARP activity, and specifically includes both compounds with intrinsic activity (drugs) as well as prodrugs of such compounds, which prodrugs may themselves exhibit little or no intrinsic activity.

One assay which may conveniently be used in order to assess the PARP inhibition offered by a particular compound is described in the examples below.

The present invention further provides a method of inhibiting the activity of PARP in a cell, comprising contacting said cell with an effective amount of an active compound, preferably in the form of a pharmaceutically acceptable composition. Such a method may be practised *in vitro* or *in vivo*.

For example, a sample of cells may be grown *in vitro* and an active compound brought into contact with said cells, and the effect of the compound on those cells observed. As examples of "effect" the amount of DNA repair effected in a certain time may be determined. Where the active compound is found to exert an influence on the cells, this may be

used as a prognostic or diagnostic marker of the efficacy of the compound in methods of treating a patient carrying cells of the same cellular type.

The term "treatment" as used herein in the context of treating a condition pertains generally to treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e. prophylaxis) is also included.

The term "adjunct" as used herein relates to the use of active compounds in conjunction with known therapeutic means. Such means include cytotoxic regimes of drugs and/or ionising radiation as used in the treatment of different cancer types.

The term "therapeutically-effective amount", as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio.

Active compounds may also be used as cell culture additives to inhibit PARP, for example, in order to radio-sensitize cells to known chemo or ionising radiation treatments *in vitro*.

Active compounds may also be used as part of an *in vitro* assay, for example, in order to determine whether a

candidate host is likely to benefit from treatment with the compound in question.

Administration

The active compound or pharmaceutical composition comprising the active compound may be administered to a subject by any convenient route of administration, whether systemically/ peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); topical (including e.g. transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g. by inhalation or insufflation therapy using, e.g. an aerosol, e.g. through mouth or nose); rectal; vaginal; parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot, for example, subcutaneously or intramuscularly.

The subject may be a eukaryote, an animal, a vertebrate animal, a mammal, a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline (e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, orangutang, gibbon), or a human.

Formulations

While it is possible for the active compound to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g. formulation) comprising at least one active compound, as defined above, together with

one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents.

Thus, the present invention further provides pharmaceutical compositions, as defined above, and methods of making a pharmaceutical composition comprising admixing at least one active compound, as defined above, together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, stabilisers, or other materials, as described herein.

The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Suitable carriers, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with the

carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations may be in the form of liquids, solutions, suspensions, emulsions, elixirs, syrups, tablets, losenges, granules, powders, capsules, cachets, pills, ampoules, suppositories, pessaries, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

Formulations suitable for oral administration (e.g. by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

A tablet may be made by conventional means, e.g. compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g. povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl cellulose); fillers or diluents (e.g. lactose, microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc, silica); disintegrants (*e.g.* sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose);

surface-active or dispersing or wetting agents (e.g. sodium lauryl sulfate); and preservatives (e.g. methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active compound therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration (e.g. transdermal, intranasal, ocular, buccal, and sublingual) may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol, or oil. Alternatively, a formulation may comprise a patch or a dressing such as a bandage or adhesive plaster impregnated with active compounds and optionally one or more excipients or diluents.

Formulations suitable for topical administration in the mouth include losenges comprising the active compound in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active compound in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active compound in a suitable liquid carrier.

Formulations suitable for topical administration to the eye also include eye drops wherein the active compound is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active compound.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebuliser, include aqueous or oily solutions of the active compound.

Formulations suitable for administration by inhalation include those presented as an aerosol spray from a pressurised pack, with the use of a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichoro-tetrafluoroethane, carbon dioxide, or other suitable gases.

Formulations suitable for topical administration via the skin include ointments, creams, and emulsions. When formulated in an ointment, the active compound may optionally be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active compounds may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example, at least about 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active compound through the skin or other affected areas. Examples of such dermal penetration enhancers include

dimethylsulfoxide and related analogues.

When formulated as a topical emulsion, the oily phase may optionally comprise merely an emulsifier (otherwise known as an emulgent), or it may comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabiliser. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabiliser(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Suitable emulgents and emulsion stabilisers include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulphate. The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations may be very low. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active compound, such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration (e.g. by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active compound in the solution is from about 1 ng/ml to about 10 μg/ml, for example from about 10 ng/ml to about 1 μg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be

prepared from sterile powders, granules, and tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the active compound to blood components or one or more organs.

Dosage

It will be appreciated that appropriate dosages of the active compounds, and compositions comprising the active compounds, can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects of the treatments of the present invention. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration *in vivo* can be effected in one dose, continuously or intermittently (e.g. in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and

the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

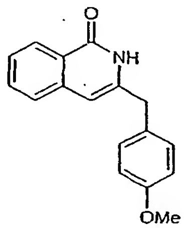
In general, a suitable dose of the active compound is in the range of about 100 µg to about 250 mg per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

EXAMPLES

The following are examples are provided solely to illustrate the present invention and are not intended to limit the scope of the invention, as described herein.

<u>Synthesis data</u>

The following compounds were synthesised using the routes set out above:



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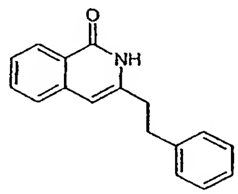

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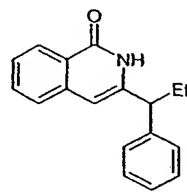

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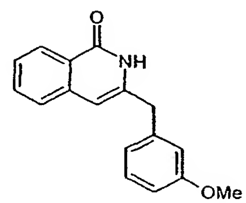

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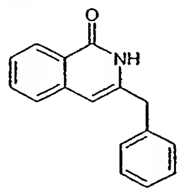

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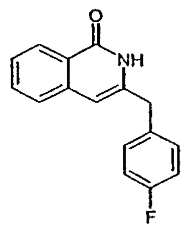

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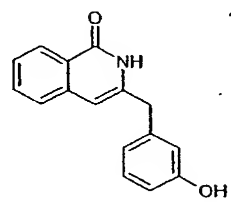

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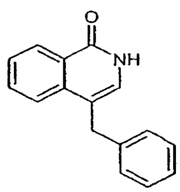

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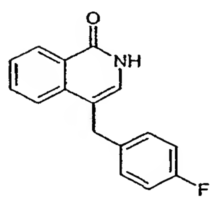

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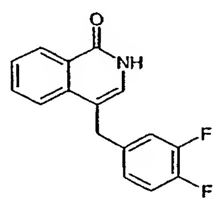

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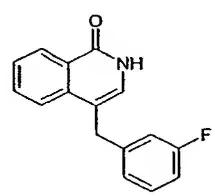

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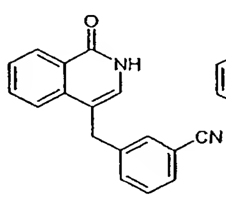

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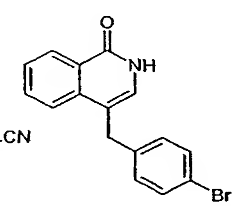

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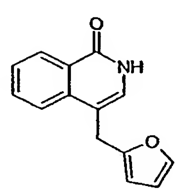

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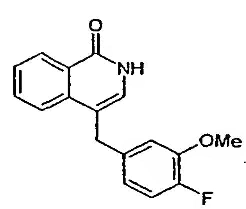

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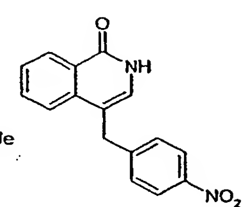


16

<u>Synthesis of 3-substituted isoquinolinones</u>

<u>Compound 1</u> (R_{C2} = 4-methoxybenzyl, R_{C1} and R_N = H)

Step 1

A well-stirred mixture of homophthalic acid (10 g; 56 mmol) and 2-(4-methoxyphenyl)acetyl chloride (10 g, 230 mmol) was heated at 200°C for 3 hours under nitrogen then cooled to

50°C and dissolved in toluene (100 ml), The solvent was removed *in vacuo* and the residue was dissolved in methanol (100 ml). Silica (30 g) was added and the solvent was removed *in vacuo*. The crude product, thereby adsorbed onto silica, was applied to the top of a column of silica and purified by chromatography using 10-50% mixtures of ethyl acetate and hexane as eluant. Appropriate fractions were combined and the solvents removed *in vacuo* to give 3-(4-methoxybenzyl)isocoumarin (0.9 g, 26%) as an oil; δ_H 3.75 (3H, s), 3.80 (2H, s), 6.50 (1H, s), 6.95 (2H, d, J = 8.9 Hz), 7.30 (2H, d, J = 8.9 Hz), 7.40-7.90 (3H, m), 8.10 (1H, d); m/z $(M+H)^{+\cdot}$ 267.

Step 2

A stirred suspension of 3-(4-methoxybenzyl)isocoumarin (0.35 g, 1.3 mmol) in 15% methanolic ammonia solution (100 ml) was heated at 150°C in a 300 ml autoclave for 5 hours then cooled to ambient temperature. The resulting solid was collected by filtration, washed with a little cold methanol and dried *in vacuo* to give 3-(4-methoxybenzyl)-1-isoquinolinone (0.04 g, 12%) as a solid; m.pt. 216-218°C; δ_H 3.80 (3H, s), 3.85 (2H, s), 6.25 (1H, s), 6.90 (2H, d, J = 8.2 Hz), 7.30 (2H, d), 7.30-7.60 (3H, m), 8.20 (1H, d), 11.20 (1H, br s); m/z $(M+H)^{+\cdot}$ 266 (100% purity).

Compounds 2-6 were prepared in a manner similar to that described above for Compound 1:

<u>Compound 2</u> (R_{C2} = 2-phenylethyl, R_{C1} and R_N = H)

Step 1

3-(2-Phenylethyl)isocoumarin. Yield, 16%; oil; δ_H 2.60-3.20 (4H, m), 6.20 (1H, s), 7.05-7.75 (8H, m), 8.25 (1H, d); m/z $(M+H)^{+\cdot}$ 251.

Step 2

3-(2-Phenylethyl)-1-isoquinolinone. Yield, 35%; m.pt. 198-200°C; δ_H 2.70-3.10 (4H, m), 6.40 (1H, s), 7.20-7.70 (8H, m), 8.20 (1H, d), 11.20 (1H, br s); m/z $(M+H)^{+\cdot}$ 250 (100% purity).

<u>Compound 3</u> (R_{C2} = 1-phenylpropyl, R_{C1} and R_N = H)

Step 1

3-(1-Phenylpropyl)isocoumarin. Yield, 14%; oil; δ_H 0.95 (3H, t), 2.10 (2H, q), 3.60 (1H, t), 6.30 (1H, s), 7.20-7.80 (8H, m), 8.20 (1H, d).

Step 2

3-(1-Phenylpropyl)-1-isoquinolinone. Yield, 43%; m.pt. 179-180°C; δ_H 0.95 (3H, t), 2.10 (2H, m), 3.60 (1H, t), 6.30 (1H, s), 7.20-7.80 (8H, m), 8.20 (1H, d), 11.10 (1H, br s); m/z $(M+H)^{+\cdot}$ 264 (97% purity).

<u>Compound 4</u> (R_{C2} = 3-methoxybenzyl, R_{C1} and R_N = H)

Step 1

3-(3-Methoxybenzyl)isocoumarin. Yield, 33%; oil; δ_H 3.75 (3H, s), 3.80 (2H, s), 6.20 (1H, s), 6.60-6.95 (2H, m), 7.10-7.65 (5H, m), 8.20 (1H, d).

Step 2

3-(3-Methoxybenzyl)-1-isoquinolinone. Yield, 45%; m.pt. 208-210°C; δ_H 3.70 (3H, s), 3.75 (2H, s), 6.35 (1H, s), 6.80-7.60 (6H, m), 8.15 (1H, d), 10.90 (1H, br s); m/z $(M+H)^{+\cdot}$ 266 (100% purity).

<u>Compound 5</u> (R_{C2} = benzyl, R_{C1} and R_N = H)

Step 1

3-Benzylisocoumarin. Yield, 60%; oil; δ_H 3.80 (2H, s), 6.15

(1H, s), 7.05-7.60 (8H, m), 8.20 (1H, d).

Step 2

3-Benzyl-1-isoquinolinone. Yield, 31%; m.pt. 193-195°C; δ_H 3.75 (2H, s), 6.35 (1H, s), 7.15-7.60 (8H, m), 8.15 (1H, d), 11.30 (1H, br s); m/z $(M+H)^{+\cdot}$ 236 (100% purity).

Compound 6 (R_{C2} = 4-fluorobenzyl, R_{C1} and R_N = H)

Step 1

3-(4-Fluorobenzyl)isocoumarin. Yield, 29%; oil; δ_H 3.80 (2H, s), 6.30 (1H, s), 7.00-7.70 (7H, m), 8.25 (1H, d).

Step 2

3-(4-Fluorobenzyl)-1-isoquinolinone. Yield, 79%; m.pt. 238-240°C; δ_H 3.80 (2H, s), 6.40 (1H, s), 7.00-7.60 (7H, m), 8.15 (1H, d), 11.30 (1H, br s); m/z $(M+H)^{+\cdot}$ 254.

Compound 7 (R_{C2} = 3-hydroxybenzyl, R_{C1} and R_N = H)

A solution of boron tribromide in dichloromethane (1M; 8.5 ml, 8.5 mmol) was added dropwise under nitrogen to an ice-cooled, stirred suspension of 3-(3-methoxybenzyl) isoquinolinone (1 g, 3.8 mmol) in dichloromethane (5ml), the stirred mixture was heated under reflux for 6 hours, then it was cooled to ambient temperature and poured onto 10% aqueous sodium hydroxide solution (30 ml). The basic solution was washed with dichloromethane (3 x 50 ml), then it was acidified by the addition of concentrated hydrochloric acid. The product was extracted into ethyl acetate (3 x 50 ml), the combined extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo* to leave 3-(3-hydroxybenzyl)isoquinolinone (0.4 g, 42%) as a white solid; m.pt. 225-227°C; δ_H 3.80 (2H, s), 6.35 (1H, s), 6.60-7.70 (7H, m), 8.10 (1H, d), 9.30 (1H, s), 11.20 (1H, br s); m/z $(M+H)^{+\cdot}$ 252 (100% purity).

Synthesis of 4-substituted isoquinolinones

Compound 8 (R_{C1} = benzyl, R_{C2} and R_N = H)

Step 1

A mixture of homophthalic acid (200 g, 1.09 mol) and urea (80 g, 1.31 mol) was ground to a fine powder then heated at 175-185°C until it had melted then resolidified. The mixture was cooled to ambient temperature, methanol (500 ml) was added, then the mixture was heated under reflux for 20 minutes, filtered, and allowed to cool to ambient temperature. The resulting solid was collected by filtration, washed with methanol and dried *in vacuo* to give homophthalimide (60 g, 34%) as a solid; m.pt. 235-240°C; δ_H 4.0 (2H, s), 7.3-7.75 (3H, m), 8.10 (1H, d), 11.20 (1H, br s).

Step 2

A stirred mixture of homophthalimide (15 g, 93 mmol), benzaldehyde (9.9 g, 93 mmol), piperidine (9 ml) and acetic acid (465 ml) was heated under reflux for 1 hour, cooled to ambient temperature and diluted with water (500 ml). The resulting solid was collected by filtration, washed with water and dried *in vacuo* to give 4-benzylidenehomophthalimide (18.5 g, 82%) as a solid; m.pt. 173-177°C. Used crude for the next step.

Step 3

Sodium borohydride (1.2 g, 32 mmol) was added in portions to a stirred suspension of 4-benzylidenehomophthalimide (2 g, 8 mmol) in methanol (50 ml), then the stirred mixture was heated under reflux for 4 hours, cooled to ambient temperature and added to water (200 ml). The product was extracted into ethyl acetate (3 x 50 ml), the combined

extracts were washed with water (2 x 30 ml), dried ($MgSO_4$) and the solvent was removed *in vacuo*. The residue was dissolved in the minimum volume of ether and sufficient hexane was added to precipitate the product. The resulting solid was collected by filtration, washed with hexane (20 ml) and dried *in vacuo* to give the 4-benzyl-3-hydroxy-3,4-dihydro-1-isoquinolinone intermediate which was used without purification.

A stirred mixture of the above intermediate (0.1 g, 0.4 mmol), toluene-4-sulphonic acid (10 mg) and toluene (50 ml) was heated under reflux for 4 hours while water was removed from the reaction by azeotropic distillation. The mixture was allowed to cool to ambient temperature, then it was washed with saturated aqueous sodium hydrogencarbonate solution (2 x 30 ml) and water (2 x 30 ml), dried ($MgSO_4$), and the solvent was removed *in vacuo* to give 4-benzyl-1-isoquinolinone (0.02 g, 3% over two stages) as a solid; m.pt. 217-220°C; δ_H 4.0 (2H, s), 7.0 (1H, s), 7.15-7.3 (5H, m), 7.45 (1H, m), 7.65 (2H, m), 8.25 (1H, d), 11.20 (1H, br s).

Compounds 9-16 were prepared in a manner similar to that described above for Compound 8 except that for Compounds 10-17, purification *via* preparative-scale high performance liquid chromatography was required for the isolation of pure material.

<u>Compound 9</u> (R_{C1} = 4-fluorobenzyl, R_{C2} and R_N = H)

Step 1

As for Compound 8

Step 2

4-(4-Fluorobenzylidene)homophthalimide. Yield, 100%; m.pt.

187-191°C; δ_H 7.1-8.2 (9H, m), 11.3-11.7 (1H, br d).

Step 3

4-(4-Fluorobenzyl)-1-isoquinolinone. Yield, 8% over two stages; m.pt. 185-188°C; δ_H 4.0 (2H, s), 6.9-7.7 (8H, m), 8.25 (1H, d), 11.20 (1H, br s); m/z $(M+H)^{+\cdot}$ 254.

<u>Compound 10</u> (R_{C1} = 3,4-difluorobenzyl, R_{C2} and R_N = H)

Step 1

As for Compound 8

Step 2

4-(3,4-Difluorobenzylidene)homophthalimide. Yield, 76%; m.pt. 199-205°C; δ_H 7.1-8.2 (8H, m), 11.3-11.7 (1H, br d).

Step 3

4-(3,4-Difluorobenzyl)-1-isoquinolinone. Crude yield, 16% over two stages; m.pt. 148-150°C; m/z $(M+H)^{+\cdot}$ 272 (31% purity). Purified by preparative scale high performance liquid chromatography on a Gilson LC unit under the following conditions: Column - Jones Chromatography Genesis 4µ C18 column, 10mm x 250mm; Mobile phase A - 0.1% aqueous TFA; Mobile phase B - acetonitrile; Flow rate 6ml/min; Gradient - starting at 90% A/10% B for one minute, rising to 97% B after 15 minutes, holding there for 2 minutes, then back to the starting conditions. Peak acquisition was based on UV detection at 254nm and compound identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode. Retention time - 4.04 minutes; m/z $(M+H)^{+\cdot}$ 272.

<u>Compound 11</u> (R_{C1} = 3-fluorobenzyl, R_{C2} and R_N = H)

Step 1

As for Compound 8

Step 2

4-(3-Fluorobenzylidene)homophthalimide. Yield, 79%; m.pt. 174-176°C; δ_H 7.1-7.6 (7H, m), 7.95-8.2 (2H, m), 11.3-11.7 (1H, br d).

Step 3

4-(3-Fluorobenzyl)-1-isoquinolinone. Crude yield, 14% over two stages; m.pt. 132-134°C; m/z $(M+H)^{+\cdot}$ 254 (37% purity). Purified by preparative scale high performance liquid chromatography on a Gilson LC unit under the following conditions: Column - Jones Chromatography Genesis 4µ C18 column, 10mm x 250mm; Mobile phase A - 0.1% aqueous TFA; Mobile phase B - acetonitrile; Flow rate 6ml/min; Gradient - starting at 90% A/10% B for one minute, rising to 97% B after 15 minutes, holding there for 2 minutes, then back to the starting conditions. Peak acquisition was based on UV detection at 254nm and compound identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode. Retention time - 3.91 minutes; m/z $(M+H)^{+\cdot}$ 254.

<u>Compound 12</u> (R_{C1} = 3-cyanobenzyl, R_{C2} and R_N = H)

Step 1

As for Compound 8

Step 2

4-(3-Cyanobenzylidene)homophthalimide. Yield, 90%; m.pt. 272-275°C; δ_H 7.3-8.2 (9H, m), 11.3-11.7 (1H, br d).

Step 3

4-(3-Cyanobenzyl)-1-isoquinolinone. Crude yield, 13% over two stages; m.pt. 85-88°C; m/z $(M+H)^{+\cdot}$ 261 (31% purity). Purified

by preparative scale high performance liquid chromatography on a Gilson LC unit under the following conditions: Column – Jones Chromatography Genesis 4µ C18 column, 10mm x 250mm; Mobile phase A – 0.1% aqueous TFA; Mobile phase B – acetonitrile; Flow rate 6ml/min; Gradient – starting at 90% A/10% B for one minute, rising to 97% B after 15 minutes, holding there for 2 minutes, then back to the starting conditions. Peak acquisition was based on UV detection at 254nm and compound identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode. Retention time - 3.55 minutes; m/z $(M+H)^{+\cdot}$ 261.

<u>Compound 13</u> (R_{C1} = 4-bromobenzyl, R_{C2} and R_N = H)

Step 1

As for Compound 8

Step 2

4-(4-Bromobenzylidene)homophthalimide. Yield, 86%; m.pt. 211-214°C; δ_H 7.2-8.2 (9H, m), 11.3-11.7 (1H, br d).

Step 3

4-(4-Bromobenzyl)-1-isoquinolinone. Crude yield, 30% over two stages; m.pt. 180-182°C; m/z $(M+H)^{+\cdot}$ 314/316 (18% purity). Purified by preparative scale high performance liquid chromatography on a Gilson LC unit under the following conditions: Column – Jones Chromatography Genesis 4µ C18 column, 10mm x 250mm; Mobile phase A – 0.1% aqueous TFA; Mobile phase B – acetonitrile; Flow rate 6ml/min; Gradient – starting at 90% A/10% B for one minute, rising to 97% B after 15 minutes, holding there for 2 minutes, then back to the starting conditions. Peak acquisition was based on UV detection at 254nm and compound identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode.

Retention time - 4.22 minutes; m/z $(M+H)^{+\cdot}$ 314/316.

Compound 14 (R_{C1} = furfuryl, R_{C2} and R_N = H)

Step 1

As for Compound 8

Step 2

4-Furfurylidenehomophthalimide. Yield, 92%; m.pt. 200-202°C; δ_H 6.7 (1H, m), 7.2-8.2 (7H, m), 11.5 (1H, br s).

Step 3

4-Furfuryl-1-isoquinolinone. Crude yield, 13% over two stages; oil; m/z $(M+H)^{+\cdot}$ 226 (48% purity). Purified by preparative scale high performance liquid chromatography on a Gilson LC unit under the following conditions: Column - Jones Chromatography Genesis 4μ C18 column, 10mm x 250mm; Mobile phase A - 0.1% aqueous TFA; Mobile phase B - acetonitrile; Flow rate 6ml/min; Gradient - starting at 90% A/10% B for one minute, rising to 97% B after 15 minutes, holding there for 2 minutes, then back to the starting conditions. Peak acquisition was based on UV detection at 254nm and compound identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode. Retention time - 3.63 minutes; m/z $(M+H)^{+\cdot}$ 226.

Compound 15 (R_{C1} = 4-fluoro-3-methoxybenzyl, R_{C2} and R_N = H)

Step 1

As for Compound 8

Step 2

4-(4-Fluoro-3-methoxybenzylidene)homophthalimide. Yield, 80%; δ_H 3.7 (3H, s), 7.0-8.1 (8H, m), 11.4-11.7 (1H, br d).

Step 3

4-(4-Fluoro-3-methoxybenzyl)-1-isoquinolinone. Crude yield, 18% over two stages; sticky solid; m/z $(M+H)^{+\cdot}$ 284 (22% purity). Purified by preparative scale high performance liquid chromatography on a Gilson LC unit under the following conditions: Column - Jones Chromatography Genesis 4µ C18 column, 10mm x 250mm; Mobile phase A - 0.1% aqueous TFA; Mobile phase B - acetonitrile; Flow rate 6ml/min; Gradient - starting at 90% A/10% B for one minute, rising to 97% B after 15 minutes, holding there for 2 minutes, then back to the starting conditions. Peak acquisition was based on UV detection at 254nm and compound identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode. Retention time - 3.84 minutes; m/z $(M+H)^{+\cdot}$ 284.

<u>Compound 16</u> (R_{C1} = 4-nitrobenzyl, R_{C2} and R_N = H)

Step 1

As for Compound 8

Step 2

4-(4-Nitrobenzylidene)homophthalimide. Yield, 94%; δ_H 7.1-8.3 (9H, m), 11.7 (1H, br s).

Step 3

4-(4-Nitrobenzyl)-1-isoquinolinone. Crude yield, 11% over two stages; sticky oil; m/z $(M+H)^{+\cdot}$ 281 (40% purity). Purified by preparative scale high performance liquid chromatography on a Gilson LC unit under the following conditions: Column - Jones Chromatography Genesis 4µ C18 column, 10mm x 250mm; Mobile phase A - 0.1% aqueous TFA; Mobile phase B - acetonitrile; Flow rate 6ml/min; Gradient - starting at 90% A/10% B for one minute, rising to 97% B after 15 minutes, holding there for 2 minutes, then back to the starting conditions. Peak acquisition was based on UV detection at 254nm and compound

identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode. Retention time - 3.85 minutes; m/z $(M+H)^{+\cdot}$ 281.

Biological Testing

In order to assess the inhibitory action of the compounds, the following assay was used to determine IC_{50} values.

Mammalian PARP, isolated from Hela cell nuclear extract, was incubated with Z-buffer (25mM Hepes (Sigma); 12.5 mM $MgCl_2$ (Sigma); 50mM KCl (Sigma); 1 mM DTT (Sigma); 10% Glycerol (Sigma) 0.001% NP-40 (Sigma); pH 7.4) in 96 well FlashPlates (TRADE MARK) (NEN, UK) and varying concentrations of said inhibitors added. All compounds were diluted in DMSO and gave final assay concentrations of between 10 and 0.01 µM, with the DMSO being at a final concentration of 1% per well. The total assay volume per well was 40 µl.

After 10 minutes incubation at 30°C the reactions were initiated by the addition of a 10 µl reaction mixture, containing NAD (5µM), ^{3}H-NAD and 30mer double stranded DNA-oligos. Designated positive and negative reaction wells were done in combination with compound wells (unknowns) in order to calculate % enzyme activities. The plates were then shaken for 2 minutes and incubated at 30°C for 45 minutes.

Following the incubation, the reactions were quenched by the addition of 50 µl 30% acetic acid to each well. The plates were then shaken for 1 hour at room temperature.

The plates were transferred to a TopCount NXT (TRADE MARK) (Packard, UK) for scintillation counting. Values recorded

identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode. Retention time - 3.85 minutes; m/z $(M+H)^{+\cdot}$ 281.

Biological Testing

In order to assess the inhibitory action of the compounds, the following assay was used to determine IC_{50} values.

Mammalian PARP, isolated from Hela cell nuclear extract, was incubated with Z-buffer (25mM Hepes (Sigma); 12.5 mM $MgCl_2$ (Sigma); 50mM KCl (Sigma); 1 mM DTT (Sigma); 10% Glycerol (Sigma) 0.001% NP-40 (Sigma); pH 7.4) in 96 well FlashPlates (TRADE MARK) (NEN, UK) and varying concentrations of said inhibitors added. All compounds were diluted in DMSO and gave final assay concentrations of between 10 and 0.01 μM, with the DMSO being at a final concentration of 1% per well. The total assay volume per well was 40 μl.

After 10 minutes incubation at 30°C the reactions were initiated by the addition of a 10 μl reaction mixture, containing NAD (5μM), ^{3}H-NAD and 30mer double stranded DNA-oligos. Designated positive and negative reaction wells were done in combination with compound wells (unknowns) in order to calculate % enzyme activities. The plates were then shaken for 2 minutes and incubated at 30°C for 45 minutes.

Following the incubation, the reactions were quenched by the addition of 50 μl 30% acetic acid to each well. The plates were then shaken for 1 hour at room temperature.

The plates were transferred to a TopCount NXT (TRADE MARK) (Packard, UK) for scintillation counting. Values recorded

identification was by mass spectroscopy on a Finnegan LCQ in positive ion mode. Retention time - 3.85 minutes; m/z $(M+H)^{+\cdot}$ 281.

Biological Testing

In order to assess the inhibitory action of the compounds, the following assay was used to determine IC_{50} values.

Mammalian PARP, isolated from Hela cell nuclear extract, was incubated with Z-buffer (25mM Hepes (Sigma); 12.5 mM $MgCl_2$ (Sigma); 50mM KCl (Sigma); 1 mM DTT (Sigma); 10% Glycerol (Sigma) 0.001% NP-40 (Sigma); pH 7.4) in 96 well FlashPlates (TRADE MARK) (NEN, UK) and varying concentrations of said inhibitors added. All compounds were diluted in DMSO and gave final assay concentrations of between 10 and 0.01 μM, with the DMSO being at a final concentration of 1% per well. The total assay volume per well was 40 μl.

After 10 minutes incubation at 30°C the reactions were initiated by the addition of a 10 μl reaction mixture, containing NAD (5μM), ^{3}H-NAD and 30mer double stranded DNA-oligos. Designated positive and negative reaction wells were done in combination with compound wells (unknowns) in order to calculate % enzyme activities. The plates were then shaken for 2 minutes and incubated at 30°C for 45 minutes.

Following the incubation, the reactions were quenched by the addition of 50 μl 30% acetic acid to each well. The plates were then shaken for 1 hour at room temperature.

The plates were transferred to a TopCount NXT (TRADE MARK) (Packard, UK) for scintillation counting. Values recorded

Cell growth was assessed using the sulforhodamine B (SRB) assay (Skehan, P., *et al.*, 1990, *J. Natl. Cancer Inst.*, **82**, 1107-1112). 2,000 HeLa cells were seeded into each well of a flat-bottomed 96-well microtiter plate in a volume of 100 μl and incubated for 6 hours at 37°C. Cells were either replaced with media alone or with media containing the test compound at a final concentration of 25 μM. Cells were allowed to grow for a further 1 hour before the addition of bleomycin to either untreated cells or test compound treated cells. Cells untreated with either bleomycin or test compound were used as a control. Cells treated with test compound alone were used to assess the growth inhibition by the test compound.

Cells were left for a further 16 hours before replacing the media and allowing the cells to grow for a further 72 hours at 37°C. The media was then removed and the cells fixed with 100μl of ice cold 10% (w/v) trichloroacetic acid. The plates were incubated at 4°C for 20 minutes and then washed four times with water. Each well of cells was then stained with 100μl of 0.4% (w/v) SRB in 1% acetic acid for 20 minutes before washing four times with 1% acetic acid. Plates were then dried for 2 hours at room temperature. The dye from the stained cells was solubilized by the addition of 100μl of 10mM Tris Base into each well. Plates were gently shaken and left at room temperature for 30 minutes before measuring the optical density at 564nM on a Microquant microtiter plate reader.

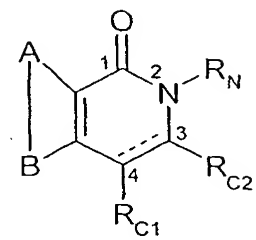
Results

IC_{50} (μM): Compound 2 - 0.58; Compound 4 - 8.5; Compound 7 - 1.8; Compounds 9-16 ≤2

DEF: Compound 1 - 1.15; Compound 2 - 1.4; Compound 4 - 1.2; Compound 5 - 1.1; Compound 7 - 1.2

CLAIMS

1. The use of a compound of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for inhibiting the activity of PARP, wherein:

A and B together represent an optionally substituted, fused aromatic ring;

the dotted line between the 3 and 4 positions indicates the optional presence of a double bond;

at least one of R_{C1} and R_{C2} is independently represented by $-L-R_L$, and if one of R_{C1} and R_{C2} is not represented by $-L-R_L$, then that group is H, where L is of formula:

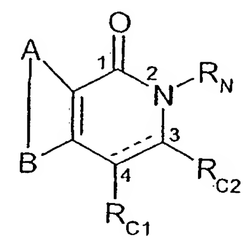
$-(CH_2)_{n1}-Q_{n2}-(CH_2)_{n3}-$

wherein n_1, n_2 and n_3 are each selected from 0, 1, 2 and 3, the sum of n_1, n_2 and n_3 is 1, 2 or 3 and each Q (if n2 is greater than 1) is selected from O, S, NR_3, C(=O), or $-CR_1R_2-$, where R_1 and R_2 are independently selected from hydrogen, halogen or optionally substituted C_{1-7} alkyl, or may together with the carbon atom to which they are attached form a C_{3-7} cyclic alkyl group, which may be saturated (a C_{3-7} cycloalkyl group) or unsaturated (a C_{3-7} cycloalkenyl group), or one of R_1 and R_2 may be attached to an atom in R_L to form an unsaturated C_{3-7} cycloalkenyl group which comprises the carbon atoms to which R_1 and R_2 are attached in Q, $-(CH_2)_{n3}-$ (if present) and part of R_L, and where R_3 is selected from H or C_{1-7} alkyl; and

R_L is selected from optionally substituted C_{3-20} heterocyclyl, C_{5-20} aryl and carbonyl; and

R_N is selected from hydrogen, optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, hydroxy, ether, nitro, amino, thioether, sulfoxide and sulfone.

2. The use of a compound of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, in the preparation of a medicament for use as an adjunct in cancer therapy, wherein:
A and B together represent an optionally substituted, fused aromatic ring;
the dotted line between the 3 and 4 positions indicates the optional presence of a double bond;
at least one of R_{C1} and R_{C2} is independently represented by $-L-R_L$, and if one of R_{C1} and R_{C2} is not represented by $-L-R_L$, then that group is H, where L is of formula:
$-(CH_2)_{n1}-Q_{n2}-(CH_2)_{n3}-$
wherein n_1, n_2 and n_3 are each selected from 0, 1, 2 and 3, the sum of n_1, n_2 and n_3 is 1, 2 or 3 and each Q (if n2 is greater than 1) is selected from O, S, NR_3, C(=O), or $-CR_1R_2-$, where R_1 and R_2 are independently selected from hydrogen, halogen or optionally substituted C_{1-7} alkyl, or may together with the carbon atom to which they are attached form a C_{3-7} cyclic alkyl group, which may be saturated (a C_{3-7} cycloalkyl group) or unsaturated (a C_{3-7} cycloalkenyl group), or one of R_1 and R_2 may be attached to an atom in R_L to form an unsaturated C_{3-7} cycloalkenyl group which comprises the carbon atoms to which R_1 and R_2 are attached in Q, $-(CH_2)_{n3}-$ (if present) and part of R_L, and where R_3 is selected from H or C_{1-7} alkyl; and

R_L is selected from optionally substituted C_{3-20} heterocyclyl, C_{5-20} aryl and carbonyl; and R_N is selected from hydrogen, optionally substituted C_{1-7} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, hydroxy, ether, nitro, amino, thioether, sulfoxide and sulfone.

3. The use according to claim 2, wherein the adjunct is for use in combination with ionising radiation.

4. The use according to claim 2, wherein the adjunct is for use in combination with chemotherapeutic agents.

5. The use according to any one of claims 1 to 4, wherein there is a double bond present between the third and fourth positions of the compound.

6. The use according to any one of claims 1 to 5, wherein one of R_{C1} and R_{C2} is represented by $-L-R_L$, and the other of R_{C1} and R_{C2} is H.

7. The use according to any one of claims 1 to 6, wherein the fused aromatic ring(s) represented by -A-B- consist solely of carbon ring atoms.

8. The use according to claim 7, wherein the fused aromatic ring represented by -A-B- is benzene.

9. The use according to either claim 7 or claim 8, wherein said rings are unsubstituted.

10. The use according to any one of claims 1 to 9, wherein R_N is hydrogen.

11. The use according to any one of claims 1 to 10, wherein

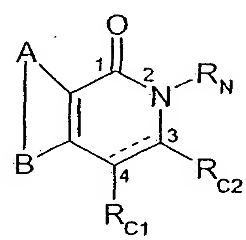
L is of the formula:
$-(CH_2)_{n1}-Q_{n2}-$, where n1 is selected from 0, 1, 2 and 3 and n2 is selected from 0 and 1, where the sum of n1 and n2 is 1, 2 or 3.

12. The use according to claim 11, wherein n1 is 1.

13. The use according to claim 12, wherein L is $-CH_2-$.

14. The use according to any one of claims 1 to 13, wherein R_L is an optionally substituted benzene ring.

15. A compound of the formula:



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, wherein:
A and B together represent an optionally substituted, fused aromatic ring;
the dotted line between the 3 and 4 positions indicates the optional presence of a double bond;
one of R_{c1} and R_{c2} is $-CH_2-R_L$, and the other of R_{c1} and R_{c2} is H;
R_L is optionally substituted phenyl; and
R_N is hydrogen.

16. A compound according to claim 15, wherein the fused aromatic ring(s) represented by -A-B- consists of solely carbon ring atoms.

17. A compound according to claim 16, wherein the fused aromatic ring represented by -A-B- is benzene.

18. A compound according to either claim 16 or claim 17, wherein said rings are unsubstituted.

19. A pharmaceutical composition comprising a compound according to any one of claims 15 to 18 and a pharmaceutically acceptable carrier or diluent.

20. The use of a compound according to any one of claims 15 to 18 in a method of treatment of the human or animal body.

INTERNATIONAL SEARCH REPORT

PCT/GB 02/01967

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D217/18 C07D217/20 C07D417/12 C07D417/14 C07D409/12
C07D407/08 C07D403/12 A61K31/47 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 355 750 A (WARNER LAMBERT CO) 28 February 1990 (1990-02-28) see generic disclosure p.5, l.30-p.6, l.18. page 3, line 1 -page 4, line 17	1-20
X	WO 91 18591 A (COLLINS MARY KATHARINE LEVINGE ;FARZANEH FARZIN (GB); SHALL SYDNEY) 12 December 1991 (1991-12-12) page 2, line 7 - line 27; claims 6-10	1-20
X	WO 99 11624 A (GUILFORD PHARM INC) 11 March 1999 (1999-03-11) cited in the application claim 1	1-20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 July 2002	02/08/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schuemacher, A

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELLIOTT I.W. JR.; TAKEKOSHI Y.: "Reduction of 4-arylidene-1,3(2H,4H)isoquinolinediones " JOURNAL OF HETEROCYCLIC CHEMISTRY, vol. 13, no. 3, 1976, pages 597-599, XP001087512 see compounds 11-14.	15-19
X	WU, MING-JUNG ET AL: "A direct anionic cyclization of 2-alkynylbenzonitrile to 3-substituted-1(2H)-isoquinolones and 3-benzylideneisoindol-2-ones initiated by methoxide addition" TETRAHEDRON (1999), 55(46), 13193-13200 , XP004180919 scheme 2,compounds 4c,4d,4g.4h.	15-19
X	WO 93 14086 A (SYNTEX , INC., USA) 22 July 1993 (1993-07-22) see abstract and claim 1.	15-19
X	JP 58 164577 A (TAIHO PHARMACEUTICAL CO., LTD., JAPAN) 29 September 1983 (1983-09-29) see in abstract, intermediate II.	15-19
X	DE 21 43 745 A (FARBWERKE HOECHST A.-G.) 8 March 1973 (1973-03-08) see intermediate III in claim 8.	15-19
X	EP 0 502 575 A (MERCK & CO INC) 9 September 1992 (1992-09-09) see intermediate 2 in scheme 2 and 4.	15-19
X,P	US 6 262 068 B1 (ATWAL, KARNAIL S. ET AL) 17 July 2001 (2001-07-17) claim 1	15-19
X	US 2 612 503 A (ULLYOT GLENN E) 30 September 1952 (1952-09-30) column 1, line 25 -column 2, line 23; example 6	15-19
X	GB 721 286 A (SMITH KLINE & FRENCH INTERNAT) 5 January 1955 (1955-01-05) column 2, line 1-55; example 4	15-19
X	JP 54 156526 A (ASAHI CHEMICAL IND) 10 December 1979 (1979-12-10) abstract	15-19

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DUSEMUND, JUERGEN ET AL: "Facile synthesis of isoquino'2,3-c!'2,3!benzoxazepinone and -'2,3!benzodiazepinones and their precursors" ARCH. PHARM. (WEINHEIM, GER.) (1988), 321(1), 41-4 , XP001087615 see compound 8	15-19
X	MODI, A. R. ET AL: "Isoquinolones - an elegant synthesis for 3-acetyl and 3-benzoylisoquinolones" CURR. SCI. (1979), 48(13), 580-1 , XP001087614 see compound III p.580	15-19
X	BELGAONKAR, VASANT H. ET AL: "Isocoumarins. XIV. Synthesis of 3-benzylisocoumarins and 3-benzyl-1(2H)-isoquinolones" INDIAN J. CHEM. (1975), 13(4), 336-8 , XP001087612 see compound VIII	15-19

INTERNATIONAL SEARCH REPORT

PCT/GB 02/01967

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1(part.),2(part.),15(part.)
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
   see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1(part.),2(part.),15(part.)

Present claims 1, 2 and 15 relate to an extremely large number of possible compounds. In fact, the claims contain so many options due to the use of teh terms "chemically protected forms and prodrugs" and possible permutations due to the use of the terms" optionnaly substituted" that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely according to the definition of the term prodrug given in the description on p.29, l.14-p.30,l.3 and the examples of the compounds mentioned in the description.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/GB 02/01967

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
EP 0355750	A	28-02-1990	AT	117553	T	15-02-1995
			CA	1334969	A1	28-03-1995
			DE	68920798	D1	09-03-1995
			DE	68920798	T2	18-05-1995
			EP	0355750	A1	28-02-1990
			ES	2067508	T3	01-04-1995
			GR	3015850	T3	31-07-1995
			JP	2124874	A	14-05-1990
			JP	2786896	B2	13-08-1998
			US	5177075	A	05-01-1993
WO 9118591	A	12-12-1991	AT	142873	T	15-10-1996
			AU	645812	B2	27-01-1994
			AU	7880491	A	31-12-1991
			CA	2082825	A1	26-11-1991
			DE	69122248	D1	24-10-1996
			DE	69122248	T2	06-03-1997
			DK	531370	T3	07-10-1996
			EP	0531370	A1	17-03-1993
			WO	9118591	A1	12-12-1991
			GB	2244646	A ,B	11-12-1991
			IE	911784	A1	04-12-1991
			PT	97754	A ,B	30-04-1992
			US	5633282	A	27-05-1997
			ZA	9103996	A	27-01-1993
WO 9911624	A	11-03-1999	US	2002022636	A1	21-02-2002
			AU	9297898	A	22-03-1999
			AU	9298098	A	22-03-1999
			AU	9298198	A	22-03-1999
			AU	9298698	A	22-03-1999
			AU	9299198	A	22-03-1999
			AU	9374898	A	22-03-1999
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